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All of our experiments utilize erythrocyte ghost membranes. Dielectrophoresis is induced with a 60 Hz (hertzs) alternating electric current passed through a membrane suspension to induce pearl chain formation (which causes membranes to come into contact with each other). A strong direct current pulse passed in the same direction to these membranes in contact causes membrane fusion to take place with a yield which is measured. The pulses are exponentially-decaying, and the pulse duration is given as a decay half-time. We use fluorescence light microscopy and the fluorescent label DiI to label the membranes and provide a rigorous and unambiguous indicator of membrane fusion. The following six findings were made during this research effort: 1. The dielectrophoretic force between two erythrocyte membranes was measured at a low frequency (60 Hz). 2. Further evidence was obtained that electroosmosis takes place during electroporation. 3. The earliest detectable evidence for membrane fusion takes place after a finite time

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interval following the application of the fusogenic electric pulse. 4. What are electrofusion yields when dissimilar membranes are the membrane substrates compared to similar membranes (similar fusions are A+A, B+B, compared to dissimilar fusions A+B). In this work, the heterofusion yield was nearly half way between the two homofusion yields. This represents a new qualitative finding which, when combined with other results may shed light on how pairs of membranes fuse when they are different compared to when they are similar. 5. There is evidence that fusion yields are modulated by biologically relevant facts in membranes. 6. The presence of macromolecular solutes at low concentration in the membrane suspension has significant effects on the fusion mechanism.

Annual Report on ONR Grant N00014-89-J-1715

PRINCIPAL INVESTIGATOR: Arthur E. Sowers

CONTRACTOR: American Red Cross (at the Holland Laboratory, Rockville, MD)

PROJECT TITLE: The Mechanisms of Membrane Fusion by an Electric Pulse

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All of our experiments utilize erythrocyte ghost membranes. Dielectrophoresis is induced with a 60 Hz alternating electric current passed through a membrane suspension to induce pearl chain formation (which causes membranes to come into contact with each other). A strong direct current pulse passed in the same direction to these membranes in contact causes many of them to fuse. Previous studies have shown that the relationship between fusion yield and the pulse characteristics has a reciprocal dependence on the product of field strength and pulse duration. As our pulses are exponentially-decaying, the pulse duration is given as a decay half-time. We use fluorescence light microscopy and the fluorescent label DiI to label the membranes and provide a rigorous and unambiguous indicator of membrane fusion.

The following is a summary of our results and a short explanation of their significance. During the funding period, a total of 33 original graphical figures of experimental measurements, two original tables, and 8 original photographic documentation groupings were either part of submitted manuscripts or were published in connection with our research activities.

I. **Measure of Dielectrophoretic Force at Low Frequency** (Dimitrov et al, 1990-accepted for publication in Biochim. Biophys. Acta.). When alternating current passes through a membrane suspension, it induces pearl chain formation and video microscopy of the alignment was analyzed in terms of the distance-time relation to derive net forces between membranes. This was significant because it was: i) the first actual measurement of the forces with low (60 Hz) frequency sine wave alternating current, ii) demonstrated that this can be performed with relative ease with which the measurements could be made, and iii) showed variability in the data which was greater than experimental error. This suggested that because different membranes had different alignment properties, this may be a practical new approach to measuring biological variability as well as membrane properties which may be useful for fusion studies.

2. **Further Evidence that Electroosmosis takes place during Electroporation** (Dimitrov and Sowers, 1990, in press). Independent data was obtained which supported the hypothesis that the phenomenon of electroosmosis plays a significant if not dominant role in the transport of material through electropores. This data was obtained as changes in trans-electropore movement were followed when the ionic strength was altered and when divalent cations were present. When the

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divalent cations were present, the amount of electroosmosis was dramatically reduced. This is consistent with the hypothesis that electroosmosis occurs during electroporation.

3. **The Earliest Detectable Evidence for Membrane Fusion takes place after a Finite Time Interval Following the Application of the Fusogenic Electric Pulse** (Dimitrov and Sowers, 1990 - submitted). In the course of routine experiments it was discovered that there is a finite, reproducible, and easily measurable time interval between the application of a fusogenic electric pulse and the earliest detectable evidence of membrane fusion. This time interval was highly dependent on pulse characteristics (field strength, duration), buffer strength, viscosity of the suspension medium, and temperature. It was not significantly dependent on the presence of residual amounts of hemoglobin remaining in the ghost membranes. Although the shortest intervals are in the same time scale as the resolution of the methodology (17 msec), the longer intervals are up to 3-5 seconds in length and therefore measurable with high precision (17 msec/1,000 msec = 1-2 %, or better). Since a similar interval has been found for enveloped virus-plasma membrane fusions, this discovery may have significant implications for the study of the mechanism of biological fusion in a relevant model system as well as for the study of the mechanism of electrofusion.

4. **What are Electrofusion Yields when Dissimilar Membranes are the Membrane Substrates compared to Similar Membranes** (Sowers, 1989b). Fusion yields which are obtained when a rabbit erythrocyte ghost is fused to a second rabbit erythrocyte ghost are higher for the same buffer conditions and pulse than when a human erythrocyte ghost is fused to another human erythrocyte ghost. These fusions represent homofusions (i.e. A+A, B+B, etc.). When dissimilar membranes are fused (i.e. A+B), then the question is what fusion yields will be obtained. Earlier work (Sowers and Kapoor, 1987) on the fusion of chicken erythrocyte ghosts showed that the heterofusion yields could be much closer to one, but not the other, homofusion yield. In this work, the heterofusion yield was nearly half way between the two homofusion yields. This represents a new quantitative finding which, when combined with other results may shed lignt on how pairs of membranes fuse when they are different compared to when they are similar.

5. **There is Evidence that Fusion Yields are Modulated by Biologically Relevant Factors in Membranes** (Sowers, 1989a). The possibility of placing membranes in a buffer and exposing them to an electric pulse offers the chance to try to track changes in the membrane by observing changes in the fusion yields. Indeed, since the buffer conditions and fusogenic pulse are identical, our observations of qualitatively similar patterns in the changes of fusion yield when ghost membranes are made from intact cells but after various periods of storage suggest such a possibility. Indeed, the observation that these changes are qualitatively identical for both rabbit as well as human erythrocyte ghosts suggests that the storage effect on these cells is similar and relevant. A future goal is to try to identify whether the alteration in fusion yield can be traced to a change in a membrane component or a membrane property, or some combination thereof.

6. The Presence of Macromolecular Solutes at Low Concentration in the Membrane Suspension has Significant Effects on the Fusion Mechanism (Sowers, 1990-submitted). We have observed that trace amounts of residual hemoglobin remaining in the erythrocyte ghosts also led to small (10-20 %) but consistently higher fusion yields. We found, in further experiments, that this effect occurred over a broad range of pulse field strengths and durations, and was independent of the method of preparing the membranes and so was not an artifact. Furthermore, the hemoglobin could be totally removed and replaced with one of two other macromolecular solutes, bovine serum albumin and 70 kDa average molecular weight Dextran, and get the same qualitative result. This shows that we have identified a new factor which has an effect on the fusion mechanism.

Publications during funding period:

Dimitrov, D.S., and Sowers, A.E. (1990-submitted) A delay in membrane fusion: lag times observed by fluorescence microscopy of individual fusion events. Biochemistry.

Dimitrov, D.S., Apostelova, M.A., and Sowers, A.E. (1990-accepted) Attraction, Deformation and Contact of Membranes Induced by a 60 Hz Sine Wave Electric Field. Biochim. Biophys. Acta.

Sowers, A.E. (1990-submitted) Low Concentrations of Macromolecular Solutes Significantly Affect Electrofusion Yield in Erythrocyte Ghosts.

Dimitrov, D.S., and Sowers, A.E. (1990-in press) Membrane Electroporation - Fast Molecular Exchange by Electroosmosis. Biochim. Biophys. Acta

Sowers, A.E. (1990) The Study of Membrane Fusion and Electroporation Mechanisms, in: M.J. Allen, S.F. Cleary, and F.M Hawkridge, eds., Charge and Field Effects in Biosystems 2, Plenum Press, New York. 315-337.

Sowers, A.E. (1989b) Electrofusion of Dissimilar Membrane Fusion Partners Depends on Additive Contributions from Each of the Two Different Membranes. Biochim. Biophys. Acta. 985,339-342.

Sowers, A.E. (1989a) Evidence that Electrofusion Yield is Controlled by Biologically Relevant Membrane Factors. Biochim. Biophys. Acta. 985,334-338.

Neumann, E., Sowers, A.E., and Jordan, C.A., eds. (1989), Electroporation and Electrofusion in Cell Biology, (Plenum Press, New York), 436 pp.

Sowers, A.E. (1989) The Mechanism of Electroporation and Electrofusion in Erythrocyte Membranes, in Electroporation and Electrofusion in Cell Biology, (E. Neumann, A.E. Sowers, and C. Jordan), Plenum Press, New York. 229-256.